

Discussion

Early eelgrass restoration efforts on the Chesapeake Bay involved transplanting adult eelgrass plants from healthy source beds to restoration locations with costs averaging \$37,000/acre excluding monitoring costs (Fonseca et al. 1998). This and other restoration methods are both expensive and labor intensive, and can damage donor beds. Despite some advantages to using adult plants, for example, successful adult plants yield reproductive shoots during the following year's reproductive season (Orth et al. 2003), broadcasting seed appears to be a more efficient and cost effective restoration technique with the added benefit of having less impact on donor beds (Orth et al. 2000). In order to meet the goals of the CBP and the SAV Strategy, MD-DNR developed this project to conduct large scale eelgrass restoration using seeds at select locations on the Patuxent River, Maryland.

Site Selection

The most important step of any restoration project is selecting the proper location. Poor site selection has been identified as a major limitation in restoration project success (Harrison 1987; Fonseca 1992). Locations have typically been based as much on logistics and practicality as on data from habitat assessments that indicate suitable conditions for SAV success. At the start of this project, much attention was given to site selection. This included the development and refinement of a modeling program designed specifically to assess large areas of Chesapeake Bay for their restoration potential. Following computer identification, the sites identified underwent a two-year

site selection process of test plantings and water quality monitoring. The Patuxent River was one such location.

The Patuxent River is one of the most monitored and modeled rivers of its size in the world. It has become an important proving ground for many of the Chesapeake Bay Program Initiatives (Maryland Department of Natural Resources 2005). Prior to the decline of SAV beds in Chesapeake Bay in the 1960's and 1970's, the Patuxent River supported diverse populations of SAV including *Zannichellia palustris*, *Ruppia maritima*, *Potamogeton perfoliatus* and *Zostera marina* (Brush and Davis 1984). Eelgrass was documented in the lower Patuxent, southwest of Solomons Island, during a 1971 ground survey (Peter Bergstrom, personal communication). The combination of documented historical eelgrass coverage, water quality meeting the SAV habitat requirements according to the SAV targeting system (Parham and Karrh 1998), and the vast water quality dataset for the Patuxent River made this river a prime candidate for large scale eelgrass restoration efforts.

Seed Collection

Harvesting of eelgrass seeds for restoration in Chesapeake Bay involved hand collection using SCUBA or snorkeling through 2003. This can be effective for small-scale restoration, but to meet the Chesapeake 2000 goal of 1,000 acres by 2008, innovative techniques for enhanced seed collection were needed. Seed collection was increased from approximately 2.3 million seeds in 2003 using hand harvesting, to approximately

15.1 and 12 million seeds in 2004 and 2005, respectively. This was due primarily to the use of a mechanical harvester in 2004 and 2005.

Sexual reproduction of eelgrass occurs when adult plants produce a reproductive shoot that extends up into the water column above the plant. When using the mechanical harvester to collect reproductive material in large quantities, a number of steps were taken to prevent harming the existing plants within the donor beds. The depth of the cutting blades on the harvester was adjusted to avoid damage to the rhizome mat and lower vegetative parts of the eelgrass plants. This prevents damage to a bed because the flowering shoots are adapted to break off and be carried away by wind and tides for the dispersal of the seeds carried within. Leaving the rhizome mat intact with some amount of vegetative material still attached allowed plants to continue to grow after cutting was performed. After the seeds fall out of the reproductive shoots, these shoots serve no further purpose in the health or continued success of the bed (Granger et al. 2002). As a precaution, harvesting took place over a large area to assure that sufficient seeds remained for bed maintenance (Granger et al. 2002). To confirm that there was no significant damage to eelgrass beds where reproductive shoots had been harvested, divers used SCUBA to survey the harvested beds 8 weeks (July 22, 2004) after the 2004 collection. Divers reported abundant, healthy eelgrass and quite a bit of flowering widgeon grass. There were no substantial differences in plant height, bed density, or apparent vigor of the plants themselves between the harvested and unharvested beds. In addition, aerial photography taken on June 19 and July 6, 2004 confirmed that the areas that were harvested in May were still densely vegetated (VIMS; 2004 field observations

and aerial photography accessible:

<http://www.vims.edu/bio/sav/2004obs.html#vims071304>).

Test Plantings

Test plantings were carried out to ensure that areas identified by the site selection model would support eelgrass growth. The plants were able to root successfully as seen by the persistence of plants from November 2004, when planted, through the winter and spring until surveyed in May 2005. The purpose of these plants is to serve as an indicator of what to expect from seedlings that emerge as a result of seedings. Seedlings would not be expected to thrive if healthy transplanted adult plants were not able to survive. The total loss of all plants at each of the test plot locations during the summer of 2005 is an indication of poor water quality during that time.

Eelgrass seeds in Restoration

Planting density effects

Restoration projects utilizing eelgrass seeds in Maryland and Virginia have been successful in the field (Orth et al. 2003). The density at which seeds are broadcast and the size and location of the plots are considered potentially important factors affecting the germination of eelgrass seeds and plant survival. Orth et al. (2003) tested five seeding densities ranging from approximately 10,000 seeds/acre to 5,000,000 seeds/acre at twelve different location baywide (both MD and VA) and found no density dependent effects on germination rate or seedling success. In the Patuxent, during the current study, seeds were broadcast at a density of 150,000 seeds/acre in 2004. Initial seed broadcasts in

August of 2005 were carried out at a density of 67,000 seeds/acre. Since the data for this report was prepared, these same areas have since been enhanced with an additional 200,000 seeds/acre resulting in a final seed density of 267,000 seeds/acre for all 2005 broadcasts. After spring surveys (May 2006) the effectiveness of different seeding densities will be closely examined to evaluate the potential for site-specific variation in density dependence.

Plot Size Effects

Some literature suggests that larger, denser, restored beds are more likely to positively influence water quality, and therefore, are less susceptible to perturbations such as storms or mute swans. Due to the physical presence of three-dimensional structure provided by SAV, and the increased “roughness” of the bottom in SAV beds, water velocities are reduced as much as 50% reduced within SAV beds (Fonseca et al. 1982; Benoy and Kalff 1999; Gacia et al. 1999). Furthermore, it has been noted that water velocity reductions are directly proportional (as a power function) to both the height and the growth form of the species that occur in the area (Petticrew and Kalff 1992; Gacia et al. 1999). However, despite reasons to expect that plot size might affect plant survival, restoration efforts with eelgrass in plots of different sizes (4 m² to 400m²) and configurations (alternating 4 m² patches and large continuous patches) in different river systems in Virginia (Virginia Institute of Marine Science) have shown a significant site effect but no significant plot size effect on germination of eelgrass seeds (Orth, personal communication).

Seed Storage Issues

Storing the spring-harvested seeds over the summer is one of the most difficult aspects of this project. Each year there has been a substantial loss of seeds during seed storage,

ultimately decreasing the number of viable seeds at the end of the storage process and reducing the acreage of SAV restored for this project. During the seed harvest of 2003, 2.3 million seeds were collected, only 250,000 (11%) of which were viable and used for broadcast. Harvest efforts in 2004 collected 15.12 million seeds. However, 1,058,400 seeds (7%) of these were deemed viable in the fall. The 2005 harvest collected 109.5 liters, 12,373,500 seeds. Unfortunately, only 2,527,000 (20%) seeds were viable at the end of the seed processing/storage procedure. After the 2004 season, biologists from VIMS and DNR attempted to identify potential problems with the seed transport and separation and holding/storage process. The lack of general research on seed physiology made identifying specific problems very difficult. In 2005, seed storage experiments were set up at St. Mary's College, VIMS, and MD-DNR to test the impact of the following parameters: flow, aeration, salinity, and stirring. When the results of these experiments are analyzed, appropriate modifications will be made to the seed processing and storage procedure to be applied in 2006.

A buoy-deployed seeding system (BuDSS) developed by Pickerell et al. (2003; 2005) was modified slightly and used as an alternative method to broadcast seeding in the fall. There are several potential advantages to using this method, most importantly, eliminating the need to store seeds during the summer. For this method, reproductive material is placed in mesh bags immediately after harvest, moved to the restoration location, and deployed in the area to be restored. Immediate deployment of reproductive material eliminates the need to store seeds, reducing the number of seeds lost to processing and decreasing the expense and labor requirements associated with seed

transport, processing, and storage. The mesh bags remain suspended at the top of the water column, allowing the seeds to develop and drop over a period of weeks. This mimics the floating and rafting of reproductive shoots during natural seeding events during the natural phenological schedule (Pickerel et al. 2003; 2005). Although not proven, it has been suggested that this method may also reduce seed predation by spreading out seed dispersal over time and through a combination of time and natural forces yield a more even distribution of seeds. There are also potential problems with this method. These bags create a navigational hazard while the mesh bags are on-site (the restoration plots with floats every 10 meters are difficult to navigate). Despite staggering seed dispersal over time, seed predators are active during this time. Any sort of spring dispersal that mimics the natural dispersal will be affected by predators. In addition, the dispersal of seeds from the bags is not random and follows a figure eight shaped pattern following the ebb and flood tides as seen by Pickerel (2003).

Determining Viability

One difficulty in using eelgrass seeds for restoration lies in determining the number of viable seeds as a proportion of total seeds being put out. Whether for use in fall seed broadcasts or spring seed bags, it is necessary to know the number of viable seeds in order to determine the recruitment rate (number of seedlings/number of viable seeds put out).

Two methods were used to count seeds, one for the spring seed bag method and one for fall seed broadcast method. At the present time there is no way to know exactly how many viable seeds are broadcast by the spring seed bag method. The number of seeds put

out in seed bags was estimated by counting seeds in four 1L replicate subsamples of reproductive material and multiplying the resulting seeds/L by the total volume of harvested material used in the construction of the seed bags. This gives us an estimate of the total number of seeds dispersed using the seed bag method. However, once the bags are deployed there is no way to collect spathes from the bags once the seeds are mature and begin falling out. Seeds are never extracted directly from the spathes to analyze each of them individually and therefore, there is no direct measure of the number of viable seeds vs. dead or non-viable seeds. Therefore recruitment is calculated from the number of seedlings recruited/the total number of seeds dispersed. A method to estimate the number of viable seeds released from spring seed bags is in the process of being investigated for application during the 2006 season.

The seed estimate for the fall seed broadcast method was made after all of the seeds had fallen from the reproductive shoots and were separated from the decaying reproductive material. This method also has some inherent sources of uncertainty. Because good seeds separate from bad seeds in water, it is necessary to drain all of the water from the seed slurry and completely mix the seed mixture before obtaining a representative sample. In addition, human error is a factor in both measuring samples out as well as the squeeze test for viability. When measuring aliquots, seeds are very sensitive to packing, creating a lot of variability between the 2 ml samples. During the squeeze test a seed is deemed viable or not viable based on physical robustness of the seed. There is considerable subjectivity in this determination as well. Efforts were made to keep the methods as uniform as possible, but because of the vast number of counts that are made it

is not feasible to use the same staff member to conduct all counts. We have not been able to determine to what degree these sources of error affect our estimates.

Eelgrass Seeding Success

None of the fall seed broadcast sites on the Patuxent River successfully recruited eelgrass seedlings. Because seeds from the same batch that were broadcast on the Potomac River, MD and at several sites in Virginia (VIMS) successfully recruited plants, it can be assumed that the seeds broadcast were indeed viable. At the Parrans Hollow and Solomons Island locations, seeds were successfully recruited in the spring seed bag plots suggesting that conditions at these locations were appropriate for seed recruitment. There was not a spring seed bag plot at Hungerford Creek, and without some means to gauge whether or not eelgrass seeds were capable of being successfully recruited, this may have been an inappropriate site for eelgrass restoration. However, this site supported the adult eelgrass plants in the test plots.

The seed bag method was successful at all locations except Solomons Island. Seed deployment through the spring seed bag method yielded an average recruitment rate of 0.046%. When reviewed on a site by site basis, recruitment rates were higher at the northernmost restoration locations than at the sites closer to the mouth of the river. There was no recruitment of seedlings at Solomons Island. Recruitment increased upriver to 0.3% at Myrtle Point, and 0.05 and 0.09% at the Parrans Hollow locations. Recruitment was highest, 0.10%, at the Jefferson Patterson Park location.

Eelgrass seed recruitment as a percentage of total seeds during natural seeding appears to always be quite low. Annual seed production ranges from 6,176 seeds/m² to 24,460 seeds/m² (Olsen 1999). However, reported seedling numbers are significantly less than the numbers of seeds produced, ranging from 5-15% of the seeds produced (Harper et al. 1965; Cook 1979; Olsen and Sand-Jensen 1994; Cabin et al. 2000; Granger et al. 2002; Orth in press). Unfortunately, our seedling recruitment results were far below this average. Researchers using seeds in experimental plantings have encountered varied success as reported above, but common outcomes include, low germination rates (Moore et al 1993), wash-out of seeds (Orth et al. 1994, Harwell and Orth 1999), and seed predation (Fishman and Orth 1996).

Germination of eelgrass seeds in Chesapeake Bay is thought to be dependent upon temperature, burial, and oxygen cues (Orth and Moore 1983; Moore et al. 1993). Incorporation of seeds into the sediments (Orth and Moore 1983; Moore et al. 1993) is essential for the start of germination. Eelgrass seeds are rapidly incorporated into most sediments and generally do not move far from where they settle under various hydrodynamic regimes (Orth et al 1994). The complexity of the bottom due to biological and physical processes appears to be important for seed retention (Luckenbach and Orth 1999). Orth et al. (1994) demonstrated that turbation of the sediment as little as 1 millimeter deep could stop an eelgrass seed from rolling and being transported away. However, deep burial can stop germination. Burial of seeds below the redox potential discontinuity prevents the developing plant from receiving light (Bigeley 1981) which may be crucial to germination.

Although not made before the seedings took place, observations by divers during the 2005 surveys suggested that the bottom at Parrans Hollow and Jefferson Patterson Park were suitable for seed recruitment. Hungerford Creek showed signs of worm populations that could increase bottom roughness for settlement of seeds. Through burrowing and bioturbation, benthic infaunal species can increase seed retention through the formation of micro-sites (Rhoads and Young 1970; Rhoads 1974). A very strong current was present causing a distinct rippling pattern in bottom sediments at Myrtle Point and Solomons Island that could act to entrain seeds.

Although a strong current causes texture in the bottom that may enhance seed recruitment, it may also act to wash out or transport seeds from the dispersal site preventing germination (Chambers and MacMahon 1994; Orth et al 1994; Harwell and Orth 1999) and even uproot new seedlings or adult plants. Although we did not measure seed predation it also appears to be an important factor in seed loss (Janzen 1971; Wassenberg 1990; Fishman and Orth 1996). However, without examining these issues closely during this project, it is difficult to base conclusions on this information.

Eelgrass Survival Related to Water Quality

Perhaps more important to this project than the low recruitment rates is the inability of the recruited seedlings to survive the summer. As we saw in May 2005, seedlings were successfully established at all sites except Solomons Island, so we know that the use of the seed bag method on the Patuxent River has the potential for successful restoration.

However, both adult test plots and seedlings completely disappeared in the summer of 2005. To determine the cause for this, the water quality data collected during this study were evaluated.

Light attenuation occurring through the water column and at the leaf surface appears to be the most important factor effecting SAV growth (Kemp et al. 1983; Wetzel and Penhale 1983; Dennison 1987). A number of studies have shown that decreased light availability affects the survival of eelgrass in particular (Wetzel and Hough 1973; Phillips et al. 1978; Kemp et al. 1983; Twilley et al. 1985; Dennison and Albert 1986). Light availability data from the continuous monitoring stations, DATAFLOW cruises, and long term fixed-station cruises were analyzed to detect trends or spikes that may help explain the lack of survival of seedlings.

Turbidity values are one measure we have to determine light availability in the Patuxent during our study period. Using the EPA requirement of 22% of surface irradiance for healthy mesohaline species SAV growth, and an application depth of 1 m, a turbidity value of 5.38 NTU was determined as the water clarity target for the Patuxent River.

When we look at the percent of the time that turbidity values exceed this limit, the difference between data at the Pin Oak Farm station and the CBL station are marked.

Turbidity exceeded this limit significantly more at the Pin Oak Station, 46%, 71% and 63% of the growing season in 2003, 2004, and 2005, respectively compared to 5%, 20%, and 25% at CBL. At both stations, 2004 and 2005 conditions were worse than 2003, with 2005 being a slightly better year than 2004.

In addition to attenuation of light through the water column, epiphytic growth on the blades of SAV can further reduce light availability (Borum 1985; Twilley et al. 1985; Burt et al. 1995). On the Patuxent River, Stankelis (2003) found the highest fouling rates at the least turbid sites downriver and the lowest fouling rates at the most turbid sites, upriver. Although turbidity was significantly less near to the mouth of the river at the Solomons Island site, this decreased turbidity may have allowed for increased epiphyte growth causing the death of the adult plants there. The persistence of the adult test plot at this site as well as the upriver site and their simultaneous die off, however, is not consistent with these observations.

The data reported here reflect the results of 2004 seeding efforts. Germination of these seeds took place in the fall of 2004. New seedlings recruited from 2004 seeding efforts were subject to 2005 spring water quality conditions. Keeping in mind the importance of light, when we look closely at the turbidity conditions occurring between our three surveys, it is evident that there were episodes of severely elevated turbidity at both of the continuous monitoring stations. At the Pin Oak station there was an extended period of time, June 29th until July 17th 2005 when turbidity exceeded the habitat requirement. This episode ended just one week before our July 27th survey. Eelgrass needs between 6 and 8 hours of photosynthetic saturating irradiance to survive (Dennison and Alberte 1986). Although it is not well documented how many days healthy plants can survive elevated turbidity and decreased light availability, it is not likely that the recruited seedlings or

adult plants could survive the prolonged periods of high turbidity such as those reflected by the continuous monitor data.

Although the DATAFLOW data present a snapshot of water quality for one given time, it is useful for comparing water quality in different regions of the river. The 2005 DATAFLOW data showed recurring elevated turbidity near the Pin Oak Farm continuous monitor location, close to the Parrans Hollow and Jefferson Patterson Park sites from June to September 2005. This information coincides with the poor water quality data from the continuous monitor station near these locations.

Light attenuation in the water column (turbidity) is strongly affected by both total suspended solids and (TSS) and chlorophyll a (Chla) in the Chesapeake Bay region (Batiuk 2002). Chla was measured and recorded by continuous monitors however, TSS data was not. In 2003, correlations between Chla and turbidity were 0.5 ($P < 0.0001$, $N = 11905$) at the CBL station and 0.48 ($P < 0.0001$, $N = 10637$) at the Pin Oak Station suggesting that changes in turbidity were due in part to changes in Chla concentrations. Both of these correlations are much higher than the values at either station in 2004 and 2005. Chla and turbidity showed very weak correlations at both stations in those years suggesting that the changes in turbidity seen in 2004 and 2005 are not likely attributable to Chla. These weak correlations suggest that suspended solids, not Chla, may be the cause of the high turbidity in the Patuxent River. However, without TSS data being collected by the continuous monitors for this time frame, we can not definitely conclude that the increased turbidity is caused by TSS.

Fixed station, monthly, water quality monitoring cruises have been conducted at eleven mid-channel stations throughout the mainstem of the Patuxent River since 1985. Both secchi depth and TSS values for 2003, 2004, and 2005 (secchi depth only), were compared to the range and mean of available data from 1985 until 2002 at Saint Leonard, Point Patience, and Drum Point. Water clarity values for 2003, 2004, and 2005 are close to the mean, and do not fall outside of the range when compared to the 20-year record available at these three stations on the Patuxent River.

In Chesapeake Bay, eelgrass is near the southernmost reaches of its distribution on the east coast of the United States. There is a well documented bimodal eelgrass growth pattern with maximum growth and a peak in biomass occurring in late May to early June. A second, less dramatic growing season occurs in mid-September and continues until water temperatures drop below 10°C sometime in November. Increasing light attenuation and water temperature (above 25°C) later in June cause decreased growth and leaf defoliation (Moore et al. 1996; 1997). The metabolic rates of eelgrass are directly affected by temperature, and if too high, can increase plant respiration high enough to kill the plant (Thom et al. 2001).

Our data are consistent with this pattern. Seedlings were present and the test plots plants were thriving in mid-May 2005, but the majority of the seedlings as well as the test plots were absent when the same areas were surveyed in July 2005. If conditions were ideal and the plants had simply undergone a summer defoliation, we would have expected to

have seen those plants again during the November survey during the fall growing period. This was not the case suggesting that the plants died rather than underwent a seasonal defoliation. Subsequent surveys of areas devoid of plants could include examination of sediment for the presence or absence of intact rhizome systems in order to make better conclusions as to the fate of recruited seedlings.

Looking at potential temperature effects during this study, thresholds for eelgrass survival of 25°C and 30°C were examined. According to the continuous monitor data at both stations, temperatures exceeded 25°C at both station in every year. At the CBL station, temperature exceeded 25°C more in 2005 than in 2003 and 2004, nearly 50% of the time. At the Pin Oak station, temperatures were above 25°C over 60% of the time in 2003, dropped in 2004, and were high again in 2005 exceeding 25°C over 50% of the time. According to the continuous monitor data at both stations, temperatures exceeded 30°C for some amount of time in all years between June and Mid-September. In 2005 at the Pin Oak Station, temperature exceeded this limit consistently throughout from mid-July through September. Although it is not well documented how many days healthy plants can survive elevated temperatures, the fact that these instances of elevated temperature coincide with elevated turbidity events makes it unlikely that the recruited seedlings or adult plants could survive.

Temperature values for 2003, 2004, and 2005 were compared to the range and mean of available data from 1985 until 2002 at Saint Leonard, Point Patience, and Drum Point. Temperatures for 2003 and 2004 are close to the mean, and do not fall outside of the

range when compared to the 20-year record available at these three stations on the Patuxent River. However, in 2005, temperatures were uncharacteristically high, falling outside of the 20-year temperature range for the months of August and September at each of the three stations, and October at Point Patience.

Cost Comparison Calculations

In an effort to find the most cost-effective restoration method, attempts were made to determine the financial investment made on a per-seed basis. To do this, the total cost of the particular method was divided by the total number of viable seeds dispersed using that method. The cost per seed put out in Maryland was \$0.02 for the spring seed bag method and \$0.34 for the fall seed broadcast. The total cost for restoring one acre was determined by multiplying the cost per seed by the specified seeding density (200,000 seeds/acre). The cost for restoring one acre was determined to be \$4,473 for the spring seed bag method and \$67,085 for the fall seed broadcast method. The large seed loss during storage (93%) is responsible for the significantly higher costs per seed and per acre using the fall seed broadcast method. If 50% of the total seeds had been retained throughout the processing and storage procedure, a total of 803,628 viable seeds would have been available for broadcast on the Patuxent River, 10.69% of total (the rest was allocated for use in Potomac River, MD and VIMS). With 803,628 seeds and the same total cost for broadcast, the cost per seed would be reduced from \$0.34 to \$0.05 dropping the cost per acre from \$67,085 to \$9,379.55. This is not an unreasonable expectation as VIMS retained 80% of total seeds as viable in 2005 (Orth personal communication).

In order to achieve the same seed cost ratio as the seed bag method (\$0.02/seed and \$4,473/acre), 5,620,000 seeds would have to broadcast on the Patuxent River, 37% of the total 15.12 million seeds collected. In order for this to occur, seed viability throughout the storage process would have to be increased significantly, and a larger proportion of viable seeds (37%) would have to be allocated to the Patuxent River than in previous years (10.69%). It seems that the high costs of processes associated with the seed broadcast method, seed processing and storage, make it significantly more expensive than then dispersing seeds using the spring seed bag method.

The recruitment success of each method was determined by dividing the total number of seeds dispersed by the number of successfully recruited plants. As stated previously, however, since there was considerable difficulty in determining the number of viable seeds, this analysis is largely speculative. The spring seed bag method yielded 874 seedlings across all spring seed bag sites locations. An estimated total of 1,910,000 seeds were dispersed using this method. Therefore the overall recruitment success for the spring seed bag method was 0.05%. The fall seed broadcast method did not yield any seedlings and regardless of the number of seeds broadcast this way; the recruitment success of that method was 0.0%.

The total cost for each method was divided by the total number of successfully recruited seedlings to determine cost per successfully recruited seedling between the spring seed bag and fall seed dispersal methods. Each seedling (874) successfully recruited using the

spring seed bag method cost \$11.15. This figure could not be calculated for the fall seed broadcast method due to the lack of successful recruitment.

Other restoration efforts using the fall seed broadcast method throughout MD (DNR) and VA (VIMS) resulted in recruitment rates as ranging from 0.5 to 14.0% in 1999 and 4.3 to 13.8% in 2000. If MD DNR recruitment rates improved to be similar to these, through modification and improvements in methods, based on the 2004 cost and the number of seeds put out, the cost per successfully recruited plant would be \$40.83-\$1.46 according to 1999 rates and \$4.75-\$1.48 according to 2000 rates.

A seed bag project being conducted concurrently in 2004 in Seaside, VA (Coastal Bays; VIMS) resulted in a recruitment rate of 1.3%. Initial restoration efforts using the BuDSS in the Peconic Estuary, NY yielded 4 % recruitment (Pickerell et al. 2003). If MD-DNR recruitment rates for the seed bag method ranged from 1.3-4.0%, based on the 2004 cost and the number of seeds put out, the cost per recruited plant would drop to \$0.39-\$0.13. The projected cost of \$0.39 per plant at a 1.3% recruitment rate would still be more cost efficient than the cost per plant using the seed broadcast with a 14% recruitment rate. Seeds were successfully recruited at the Solomons Island location in 1999 by VIMS, however no plants were recruited during the 2004 seeding. Upon comparing 1999 and 2004 seeding efforts (both fall seed broadcast and spring seed bag) at several locations, it is clear that 2004 success rates are far lower than those achieved in 1999, another indication of poor water quality in 2004. Regardless of which method is used, it is abundantly clear that we must work to improve recruitment dramatically and we must

increase the density of our seeding in order to more closely mimic the density of naturally occurring eelgrass beds.

Project Website

During this year of this project, a website designated to this project was created with the hopes of educating the public about the importance of bay grasses as well as increasing awareness of current efforts to restore bay grasses in the Chesapeake Bay. The WebTrends report produced by MD-DNR Information Technology Services indicated that the website is well utilized as an educational tool. These data will be reevaluated in 2006 for long term trends in usage. The data and associated figures presented in this report will be incorporated into the website upon submission of this report. This and future reports will also be available for downloading in PDF format from the webpage.

http://www.dnr.state.md.us/bay/sav/restoration/pax_gen_info.asp

Other Problems

One of the components of the proposed work included epiphyte strip deployment, and three types of predator exclosures at each restoration site. Epiphyte strips are mylar strips developed by Dr. Walter Boynton (University of Maryland) that provide a relative, but quantitative measure of the amount of epiphytic fouling on simulated SAV blades (Stankelis et al. 1999). Epiphyte data was collected but was not analyzed in time for inclusion in this report. The results of these experiments will be included in the 2006 final report.

Several SAV predators have been demonstrated to cause damage to or destroy small-scale SAV restoration plots throughout Chesapeake Bay, including the Patuxent River. One reason for moving to large-scale SAV restoration is that large plots may be better able to withstand a small or moderate amount of predation that would destroy smaller plots. To test the effects of predation, four levels of predator enclosure were to be tested at each site: no enclosure, mute swan enclosure (surface), cownose ray enclosure (bottom), and both mute swan and cownose ray enclosure (surface and bottom). However, because of the lack of substantial numbers of recruited seedlings, these trials were delayed until a sufficient number of seedlings are established.

Similarly, this project was to determine whether the created eelgrass beds are expanding through vegetative propagation and/or natural seeding. When a sufficient number of eelgrass seedlings are established, the seed plots and surrounding area will be surveyed in the spring and fall following seeding using aerial overflights and groundtruthing with a handheld mapping GPS.

Changes in 2005 Seeding

Because of the higher success we saw at the Parrans Hollow and Jefferson Patterson Park locations, the majority of the 2005 seed dispersal efforts were concentrated near these restoration locations. In addition, seeding density was increased to between 200,000 and 267, 000 seeds/acre. All previous efforts used between 100,000 and 245,000 seeds/acre. In 2003, Orth et al. were able to demonstrate that seed-seed interactions do not affect

germination when dispersing seeds at increased densities. Thus seeding at higher densities could increase seedling production.

Continued Research and Monitoring Needs

Throughout the first two years of this project several research needs have emerged.

There is a lack of general research about seed physiology. Increased knowledge about the physiology of eelgrass seeds would help to solve ongoing problems with seed processing and storage. Similarly, a more reliable and universally accepted means of determining the number of total seeds and the percent of those seeds that are viable is needed. With poor water quality continuing to be of major concern, it is important to start to compare the resiliency of plants to different light and temperature regimes. Information such as this would help us to better understand the tight coupling between temperature and light effects that now exists. Lastly, field methods for monitoring projects such as this one could be modified to include examining the sediment for rhizomes to confirm total disappearance vs. characteristic defoliation of eelgrass plants in the summer. This information would be helpful to determine whether disappearance of plants is due mainly to death or natural dieback of the above ground plant material.

Conclusions

It is clear that water quality, specifically turbidity and temperature, as well as epiphyte loading are all potential causes for the lack of success of this project thus far. Because we were able to successfully recruit eelgrass seedlings at all but one site, using spring seed bags, we are confident that our methods are suitable for utilizing the spring seed bag

method for large scale restoration in Maryland. However, if we expect these seedlings to be successful and survive, we need to continue to increase water quality. In addition we are now aware of several technical issues that we can address to increase the success of our methods. We have begun to and will continue to address problems with seed processing, seed loss during storage, and seed counting.

Even with the technical problems causing a large loss of seeds, and the loss of all recruited seedlings that occurred, our understanding of the physical and chemical processes of this river as well as eelgrass plants and seeds have increased significantly. This report details a number of problems solved, essential information uncovered, as well as changes made for future harvest/storage/broadcasts.

It is important that we keep in mind that we expect year to year variation in eelgrass population size and growing conditions, and therefore varied success of new seedlings. With this in mind, it is still likely that if significant numbers of plants can be established in dense, protected beds, the combination of physical protection and the benefits self-protection may enable the establishment of substantial areas of eelgrass habitat within the Patuxent River.